EXPERIMENTAL RESEARCHES UPON THE PRODUCTS OF THE TUBERCLE BACILLUS.

By ANGELO MAFFUCCI, M.D.,

OF PISA,

PROFESSOR OF PATHOLOGICAL ANATOMY IN THE UNIVERSITY OF PISA
ITALY.

THE most cursory examination of a tuberculous individual shows two facts: localized inflammatory processes and general wasting.

Hitherto all inquirers have believed that the inflammatory and necrobiotic products came from the presence of the specific bacillus, and I have been convinced by experiments with the fowl-embryo (*Riforma medica*, 1889) that the general wasting is caused by the protoplasm of the living bacillus which has been destroyed by the embryos, or by the same protoplasm of the bacillus dead before inoculation into the embryos which destroyed it, because the embryos which have destroyed the living or dead bacillus were born marasmic and died.

In 1890 I got another proof: observing that the sea-hogs inoculated with dead tubercle bacilli into the cellular subcutaneous tissue died marasmic, with manifestations of abscesses at the point of inoculation, with hyperæmia in the lungs under the form of catarrhal pneumonitis, with endobronchitis proliferans and with hæmorrhagia, with nephritis parenchymalis and interstitialis, with atrophy of the liver and degeneration of the muscular fibre of the heart, with atrophy of the spleen and pigmentation. The death of these animals happened from a few days to a month after the inoculation. By these facts I could interpret the wasting and also the other inflammatory and degenerative lesions, which I found in the tissues of the tuberculous without finding tuberculosis in these organs, and, therefore, I concluded that in the pro toplasm of the dead bacillus exists a toxic substance capable of producing inflammatory and necrobiotic processes, which circulates in the blood from the inoculation-point or from a tubercular focus, destroying the red corpuscles of the blood and producing in the parenchyma of the organs atrophy, necrosis, and inflammation. Having proved these facts, I wished to know if this toxic substance, generated by the destruction of the protoplasms of the tubercle bacillus, might be influenced by the age of the bacillus, by the different grades of the temperature, by desiccation, by the sun, by the juices of the stomach, and by the different points where the inoculation, of the dead bacilli have been made.

Bacillus of Human Tuberculosis; Age of the Cultures.—The cultures of six months, of one year, of eighteen months, of two or three years, kept in humidity, do not lose the toxic power, when inoculated, in little or large doses, in the cellular subcutaneous tissues in the belly cavity and trachea of sea-hogs.

Action of the Temperature.—The recent cultures and the oldest ones, of one or two years, subjected for one or two hours to a heat of 65°, 70°, 80°, 90°, 100°, 110°C. do not lose their toxic power.

The same young cultures, kept in the oven at 45° for twenty-five days, lose their power of growth and become toxic, and are similar to old or young cultures kept under a high temperature.

Action of Desiccation.—Placing young and old tubercular cultures under a bell with air completely dry, and removing the culture medium and placing the cultures on watch-glasses for three until fourteen months, and inoculating them into sea-hogs, they maintain their toxic power.

Action of the Sunlight.—The old and young cultures, kept from fifteen days until a month on the culture medium, exposed to the sunlight with a temperature of 32°, did not lose their toxic power when inoculated into sea-hogs.

Action of the Sunlight and of the High Temperatures.—Other cultures placed until forty-five days under the action of the sunlight, and then exposed to a temperature of 100° for two hours, did not lose the toxic power when inoculated into the sea-hogs.

Action of the Digestive Juices.—Feeding the sea-hogs upon boiled tuberculous organs or sterilized cultures, they died by marasmus, as if they had been inoculated with a sterilized culture in the subcutaneous cellular tissues. Then a tubercular culture has been introduced into the stomach of a dog and then inoculated into the cellular tissue of the sea-hogs, which died of marasmus but not with tuberculosis. This fact proves that the stomach juice has no influence over the toxic product of the tubercle bacillus.

Is the toxic product of the tubercle bacillus generated by the destruction of the bacillus? A toxic product of a bacterium can be a product of secretion or of disintegration of the culture medium, on which the bacillus lives, or a product of its dead protoplasm. I have been convinced that probably the toxic product of the tubercle bacillus proceeds from the protoplasm of the dead bacillus.

Taking the cultures on liquid strata, the bacillus vegetates on the surface of the liquid, which was the blood serum of the cow, and was filtered after the method of Chamberland, and reduced under a pneumatic bell to a sixth part of its volume, and then inoculated into the sea-hogs (six centimetres equal to thirty centimetres of the liquid serum). The sea-hogs did not die by marasmus, but those inoculated with a piece of the bacillar pellicle (sterilized) died by marasmus.

These cultures diluted in distilled water with 0.75 per cent. of chloride of sodium, under the temperature of 39° for five days, and filtered and reduced in volume under a pneumatic bell, and inoculated in large doses into sea-hogs, these died by marasmus.

These experiments prove that it is not a product of disintegration of the culture medium, nor a product of secretion, and must be always an integral part of the bacillus.

Fowl Tuberculosis.—It was necessary to know if the toxic products of the bacillus of the fowl tuberculosis may be different from that of the tuberculosis of the mammalia.[1]

Action of Heat.—The cultures of the fowl tuberculosis in contrast with those of the mammal tuberculosis can develop at 25° or 45°. Therefore I made experiments with the cultures of fowl tuberculosis, grown at different temperatures, keeping these cultures for some days in the oven, or for a longer time. All the sea-hogs inoculated with those two varieties of cultures became marasmic. Keeping the old and young cultures at 100°, the inoculated sea-hogs died marasmic.

Age of the Cultures.—A characteristic of the bacillus of the fowl tuberculosis is the fact that it is destroyed in the culture media after a certain time.

The bacillus of the fowl tuberculosis grows well in liquid media without forming the pellicle characteristic of the bacillus of human tuberculosis. Therefore these growths contain more destroyed bacilli, the greater may be their age, and all the inoculated sea-hogs die by marasmus, but the old cultures make the sea-hogs die quicker,—*e.g.*, the cultures of three years make them die after a few days. This proves that the age has no importance for the diminution of the toxic power of the cultures, but shows that this power increases by the destruction of the bacillus, and therefore the oldest cultures are the most toxic.

Action of the Digestive Juices.—The sea-hogs, which eat cultures of fowl tuberculosis, die after thirty or forty days by marasmus.

Influence of the Sunlight.—Cultures of different age, kept until forty-five days under the action of the sunlight with a temperature of 32°, and inoculated into sea-hogs, make them die marasmic.

Action of the Sunlight and High Temperature.—The same cultures of different age, kept for forty-five days under sunlight, and with a temperature of 100° for two hours, inoculated into sea-hogs, make them die by marasmus.

Remarks.—My experiments and those made by Prudden,

---

[1] Riforma medica, 1889–90.

Strauss, Gamaleia, upon the dead bacillus of tuberculosis, may prove that the inflammatory forms with the anatomical characters of tubercle, may be produced by the same dead bacillus. Therefore these toxic products of the dead bacillus have a great importance, and merit all the interest of inquirers to get a possible therapic influence upon the tubercular process.

With these researches I have proved my first idea, that *the dead tubercle bacillus contains a toxic product which has a great resistance against very potent physical agents.*